The effort required to annotate and characterize the *Drosophila* genome will be proportional to the genome's size and the number of genes. Current estimates, from extrapolation of partial sequence data, give a total size of 140–150 Mb. Predicting the number of genes is a greater challenge — one that is at the heart of the annotation studies. Depending on the assumptions used, extrapolations based on the relative number of polytene bands, vital genes, predicted genes and DNA content in the *Adh* region lead Ashburner *et al.* to estimates ranging from 8,600 to almost 17,000 genes, bracketing a previous estimate[4] of 12,000 genes. The *Adh* region was, in fact, the subject of a recent annotation competition[5]. Most of the participants predicted between 20 and 30 more protein-coding genes than the 218 found by Ashburner *et al.*, highlighting the importance of functional studies in validating the predictions from bioinformatic approaches. But even if *Drosophila* turns out to have 10% more genes than the current predicted total, it will still probably have fewer than the 19,000 genes in the nematode worm *Caenorhabditis elegans*. This is surprising, given that flies are more complex than worms at many levels. To understand this obvious case of 'less is more', we will need to unravel the functional genomics of both organisms.

The community of *Drosophila* researchers is well positioned to do the functional studies that will be required to meet the challenges presented by release of the genomic sequence. The impressively large number of genes with disparate functions that map to the *Adh* interval reflects the extraordinary number and diversity of laboratories that use *Drosophila* as an experimental system. The *Drosophila* community extends well beyond those who focus on developmental genetics, and includes researchers whose interests and technical abilities embrace such diverse disciplines as biochemistry, cell biology, population studies and evolution. With the complete *Drosophila* gene list and gene knockouts, we can expect that this little bug, whose biology began genetics in this century, will be many times more useful in the next. ■

Kenneth C. Burtis and R. Scott Hawley are in the Department of Genetics, Section of Molecular and Cellular Biology, University of California, One Shields Avenue, Davis, California 95616, USA.
e-mails: shawley@netcom.com
kcburtis@ucdavis.edu

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Apoptosis

Condensed matter in cell death

Naoufal Zamzami and Guido Kroemer

Since the nineteenth century, anatomists and pathologists have observed strikingly condensed nuclei with clumped, condensed chromatin (DNA complexed with proteins) as a sign of cell death. This phenomenon, termed 'pyknosis', is often accompanied by 'karyorrhexis' — fragmentation of the nucleus. Today, at the twentieth *fin de siècle*, these terms lose some of their mystery. For on page 168 of this issue, Sahara *et al.*[1] report the molecular characterization of a hitherto unknown factor, Acinus (for 'apoptotic chromatin condensation inducer in the nucleus'). This factor seems to be indispensable for nuclear pyknosis, at least in some experimental settings.

Nuclear pyknosis and karyorrhexis are near-to-definitive hallmarks of programmed cell death (apoptosis). In teleological terms, apoptosis is a type of death designed specifically to disintegrate cells in an ordered fashion, avoiding pathogenic spillover of the cell's soluble components into tissues. This is done by maintaining the barrier function of the plasma membrane. Nevertheless, there are subtle changes in this membrane, causing neighbouring cells to engulf the dying cells. So, the doomed cell is degraded within the limits of a vesicle — the phagolysosome — of another healthy cell.

To facilitate such engulfment, apoptotic cells reduce their volume. They pump out ions (mainly K^+) and contract their reorganized cytoskeleton, which forms a sort of cage around the nucleus. Overall shrinkage is usually accompanied by nuclear pyknosis. Cells may also detach parts of their cytoplasm, which sometimes includes highly condensed fragments of the karyorrhectic nucleus. The size and membrane characteristics of these 'apoptotic bodies' makes them very palatable to neighbouring healthy cells. The dying cells also activate catabolic enzymes that ensure digestion of critical cellular components from the inside (as opposed to enzymes in the phagolysosome, which have to act from the outside). Such catabolic hydrolases include a class of specific protein-cleaving enzymes (caspases), as well as DNA-digesting enzymes (DNases), both of which participate directly or indirectly in nuclear pyknosis.

Which factors are responsible for chromatin condensation and pyknosis? In most instances, addition of caspase inhibitors prevents pyknosis. So, caspases may act upstream of other effectors, or they may act directly on nuclear proteins to dismantle the nuclear envelope and facilitate the condensation and digestion of chromatin. Sahara *et al.*[1] now suggest that caspase-3 cleaves Acinus, which is the precursor of a chromatin-condensation factor. Cleavage of Acinus at a specific aspartate residue (Asp 1093) by caspase-3 is necessary — but not sufficient — to activate the DNA-condensing activity of Acinus. For full activation, an additional (unknown) protease cleaving at serine 987 (Ser 987) has to intervene. Only the combined action of both proteases generates the mature Ser 987 to Asp 1053 fragment, which, when added to purified nuclei in the presence of unidentified nuclear-import factors, causes chromatin condensation *in vitro*.

Although Acinus is not the only factor to condense chromatin during apoptosis (Table 1), Sahara and colleagues show that it has a unique property — it does so in the absence of DNase activity. Another chromatin-condensation factor, the caspase-activated DNase (CAD), for example, uses its DNase activity to cleave chromatin at the boundaries between nucleosomes (the histone–DNA complexes that make up chromatin). In this way, CAD generates stretches of DNA about 200 base pairs (or multiples thereof) long[2]. The CAD protein is also activated by caspases, albeit indirectly. When caspase-3 or caspase-7 digests its inhibitor (ICAD), CAD is released from suppression and translocates to the nucleus. There it interacts with histone H1 and high-mobility-group 2 proteins, and exerts its DNase and chromatin-condensation activities[2]. So, as a general rule, caspases activate non-caspase effectors that participate in nuclear apoptosis[3] (Fig. 1, overleaf).

Does chromatin condensation, then, always require a caspase-dependent step? The answer is clearly no. For instance, partial or complete chromatin condensation is observed when the tumour-suppressor gene PML is overexpressed[4], or when the HIV-1 co-receptor CXCR4 is crosslinked[5] — even

Table 1 **Nuclear effects of apoptosis-promoting molecules**

	Acinus	*CAD*	*AIF*	*L-DNase II*	*Cath B*	*DNase I*	*DNase II*	*Cyclophilin*	*Caspases*
Type of chromatin condensation	Karyorrhexis	Karyorrhexis	Peripheral	Pyknosis	Peripheral	Pyknosis	Pyknosis	?	None
Type of DNA fragmentation	None	Oligos	≥50 kb	Oligos	Oligos	Oligos	≥50 kb	None	
Proteolysis	?	None	None	None	?	None	None	None	

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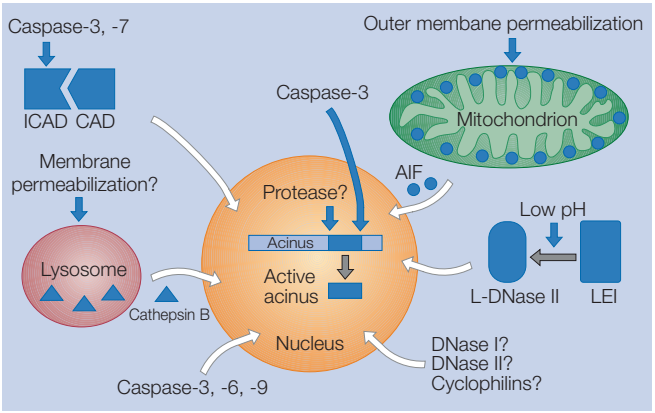

Figure 1 **Apoptotic effectors that act on the nucleus. Disruption of intracellular compartmentalization (which is well established for mitochondria but putative for lysosomes), or pH-driven conformational change, culminates in the nuclear import of effector proteins — such as caspase-activated DNase (CAD), apoptosis-inducing factor (AIF), cathepsin B, L-DNase II — or in the activation of Acinus, which is already present in the nucleus. Sahara *et al.*[1] report that cleavage of Acinus by caspase-3 and an unknown protease generates an active fragment that can cause chromatin condensation, despite lacking DNase activity. It is not clear which pathways co-activate and/or require Acinus for chromatin condensation. Other proteins with DNase activity, which are thought to be involved in apoptotic digestion of chromatin, are also listed.**

in the absence of caspase activation. Similarly, inactivation of the caspase-3 gene does not prevent chromatin condensation. Rather, it modifies the pattern of the process, or retards it according to different cell types or stimuli[6,7].

One of the proteins responsible for caspase-independent chromatin condensation has, in fact, been identified. Known as the apoptosis-inducing factor (AIF), it is a flavoprotein that is normally confined to the space between the outer and inner mitochondrial membranes[8]. Once apoptosis is induced, AIF translocates from the mitochondrion to the nucleus, where it causes partial chromatin condensation in the periphery of the nucleus[8]. This pattern of condensation is clearly distinct from those induced by CAD (ref. 3) or Acinus[1]. AIF causes degradation of DNA into fragments greater than around 50 kb in length[8] — a large-scale DNA fragmentation that precedes the more advanced internucleosomal degradation.

Another chromatin-condensation factor, which translocates from the cytoplasm to the nucleus, is L-DNase II (Fig. 1). This protein is derived from a serpin-like protease inhibitor, leukocyte elastase inhibitor, through a post-translational modification that may be triggered by cytosolic acidification[9], a metabolic change that often accompanies apoptosis. When added to purified nuclei, the L-DNase II causes a marked chromatin condensation and chops the chromatin into nucleosome-sized fragments[9]. Yet another protein that might contribute to chromatin condensation and internucleosomal DNA fragmentation is cathepsin B. This protein could be activated on its release from lysosomes of the apoptotic cell[10]. Alternatively, cathepsins might act as degradative enzymes in the phagolysosome.

Why are there so many chromatin-condensation factors? One possibility is sequential action. For example, AIF may act before the caspase-dependent chromatin-condensation factors[8]. Another idea is complementarity, which is evident from the many different effects of the distinct apoptosis-promoting molecules that act on the nucleus (Table 1). Finally, redundancy is suggested because apoptotic extracts can contain CAD and other chromatin-condensation factors[3], including Acinus[1]. Redundancy is underlined by the observation that, in Jurkat cells, overexpression of a caspase-3-resistant ICAD mutant prevents CAD-mediated DNA fragmentation, yet fails to abolish (Acinus-mediated?) chromatin condensation[11]. Some chromatin-condensation factors such as CAD are expressed in a strictly tissue-specific fashion[12], indicating that chromatin condensation may result from distinct molecular processes in different cell types. This means that, although they are morphologically uniform, pyknosis and karyorrhexis may result from highly specialized processes. Part of the mystery remains to be pierced. ■

Naoufal Zamzami and Guido Kroemer are in the Apoptosis, Cancer and Immunity Laboratory associated with the National League against Cancer, CNRS-ERS1984, 19 rue Guy Môquet, F-94801 Villejuif, France.
e-mail: kroemer@infobiogen.fr

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Daedalus

Weed insulation

Last week Daedalus proposed a ship coated with ice. Not only would it have the lowest possible skin-friction; fouling organisms could never grow on its hull. He now doubts this latter assertion. No marine plant is known to anchor itself to ice. But many specialized bacteria can live in ice, and tundra lichens can survive in an environment dominated by it. So perhaps a seaweed exists, or can be bred, to flourish on an icy surface.

Many seaweeds anchor themselves to solid surfaces, both naval and natural. They do so, not to draw nourishment from the surface, but to contact a large volume of water as it sweeps past their anchorage. A seaweed that could fasten itself to ice would gain the same benefit. DREADCO biologists are now trying to create one.

The trouble, of course, is that natural ice is usually melting. No plant can stick to its surface; it needs to carve out a cavity. Daedalus recalls that Arctic fish, like many other cold-adapted species, generate high internal concentrations of special anti-freeze compounds. If the genes for such a compound could be transferred to seaweed, and the plant could learn to exude it from its anchoring foot, it could melt out a secure root-cavity in the ice. As fast as the ice melted, it would deepen and renew its cavity. It could spread as a permanent coating on the submerged surface of an ice shelf, even colonizing the underneath. Enough light would penetrate the ice to sustain photosynthesis.

This challenging biological project has a bold ecological purpose — to reduce the melt-rate of the polar ice. During the Second World War, an artificial iceberg was proposed as an aircraft-carrier. To reduce its melt-rate, it was to be made from a dilute suspension of wood-pulp in water. This would give it a furry surface, which would be an excellent thermal insulator. Similarly, the Arctic and Antarctic polar ice shelves could be insulated by seeding them with Daedalus's ice-weed.

A menace of global warming would thus be countered. Released in the polar oceans, ice-weed would spread luxuriantly under the polar ice shelves and submarine glaciers, reducing their rate of melting. Their contribution to the rising sea-level would be cut right back. They would also release fewer free-floating icebergs to menace the world's shipping lanes. The bergs that were released would, of course, be thoroughly weed-insulated, and would last much longer. They might even be captured and towed into tropical ports for use as sources of fresh water. **David Jones**

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